ÄKTA*design*

ÄKTAbasic 10 XT upgrade kit

User Manual

18-1120-23

Amersham Biosciences

Important user information

Meaning: Consult the instruction manual to avoid personal injury or damage to the product or other equipment.

WARNING!

The Warning sign is used to call attention to the necessity to follow an instruction in detail to avoid personal injury. Be sure not to proceed until the instructions are clearly understood and all stated conditions are met.

CAUTION!

The Caution sign is used to call attention to instructions or conditions that shall be followed to avoid damage to the product or other equipment. Be sure not to proceed until the instructions are clearly understood and all stated conditions are met.

Note

The Note sign is used to indicate information important for trouble-free or optimal use of the product.

Should you have any comments on this instruction, we will be pleased to receive them at:

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SE–751 84 Uppsala
Sweden

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About this manual

This manual comprises two parts; a practical part (sections 1 – 5) and a reference part (sections A – C).

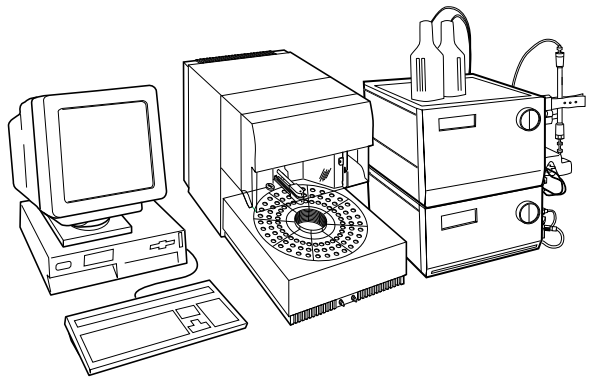
Sections 1 – 5 contain the necessary information for installing, operating and maintaining the XT upgrade equipment.

1 Introduction

1.1 General

ÄKTA™basic 10 XT upgrade kit is used for upgrading ÄKTAbasic 10 chromatography system with:

- Autosampler A-900
- Strategy, templates and adviser to support automatic sample application performed under UNICORN™ 3.0 command.
- UNICORN 3.0 Analysis module that extends the UNICORN 3.0 Evaluation module functions.



After installation and verification, your ÄKTAbasic 10 chromatography system is extended to perform the following functions:

- Automated multiple sample injections.
- Quantify amount, concentration and molecular size.

Strategy, templates and adviser

The following strategy and templates are new:

- Strategy B10X**
  Allows UNICORN to control the Autosampler A-900.
- Template Aut_y_zz
  Allows autosampler functions to be utilised in an advanced method.

In the column adviser, the column recommendations are modified to suit the columns when autosampling with A-900 is used. In the method adviser support for programming standard protocols for the B10X** strategy is included.

**=version number

UNICORN 3.0 features

Upgrading to UNICORN version 3.0 offers the possibility to utilise the UNICORN Analysis module, which allows you to perform:

- External standard quantification.
- Internal standard quantification.
- Standard addition.
- Recovery calculations.
- Molecule size calculations.

Quantification uses peak data from standards to produce calibration curves which can then be used to evaluate the amount and concentration of components in a sample.

The molecular size function determines the molecular size of components in a sample using a molecular size curve prepared from one or more standards.

1.2 Safety

- The instrument is designed for indoor use only.
- Do not use in a dusty atmosphere or close to spraying water.
- Operate in accordance with local safety instructions.

WARNING! The Autosampler A-900 must not be opened by the user. It contains high voltage circuits which can deliver a lethal electric shock.

WARNING! When using hazardous chemicals, avoid spillage during autosampling, and when the capillaries are removed.

WARNING! When using hazardous chemicals, all suitable protective measures, such as protective glasses, must be taken.

WARNING! The Autosampler A-900 must be connected to a grounded mains socket.

WARNING! Do not operate the A-900 with the front cover open.

WARNING! For continued protection against risk of fire, replace only with fuses of specified type and rating. See Technical specifications for fuse data.

The A-900 contains the following safety symbol:

SHARP NEEDLE
AND MOVING PARTS
KEEP HANDS CLEAR

This sticker denotes the proximity of the sharp needle and the moving needle arm of the A-900, which, if not handled with care, could result in injury or damage or destruction of these parts.

2 Installation

2.1 Unpacking

Unpack the ÄKTAbasic 10 XT upgrade kit, and check the items against the supplied packing list. Inspect the items for obvious damage which may have occurred during transportation.

Unless already done, unpack the Autosampler A-900 according to the installation description given in the Autosampler A-900 User Manual.

It is recommended to retain all packing material if onward transport of the Autosampler A-900 is expected.

CAUTION! Before using the A-900 for the first time, remove the safety screw marked with red tape from the bottom of the Autosampler, and the red cap from below the front cover. Allow the instrument to acclimatise for 1 hour.

CAUTION! Always lift the A-900 by the base unit, NEVER by the front cover.

CAUTION! The following information should be read carefully to ensure that the ÄKTAbasic 10 XT upgrade kit is installed correctly.

2.2 Pre-requisites

Hardware pre-requisites

The ÄKTAbasic 10 chromatography system must be installed and fully tested before the ÄKTAbasic 10 XT upgrade kit, and the Autosampler A-900 are installed. See the Installation Guide for ÄKTAbasic 10.

Software pre-requisites

The computer connected to your ÄKTAbasic 10 chromatography system must be upgraded to run UNICORN 3.0 or higher, together with new strategy, templates and adviser. The UNICORN Analysis module software should also be installed, prior to the installation test.

Follow the instructions given in the supplied software upgrade information and manuals, which are included in the ÄKTAbasic 10 XT upgrade kit.

- General installation instructions for UNICORN software are described in the UNICORN User Manual, Chapter 13. Specific upgrade information for UNICORN 3.0 or higher is given in the attached UNICORN Upgrade Instruction. Remember to check the boxes for loading strategy, templates and adviser.
- Installing the UNICORN Analysis module software is described in the UNICORN Analysis module User Manual, Section 1.4.

Install and upgrade the software before installing the hardware.

CAUTION! Make sure the ÄKTAbasic 10 chromatography system and the computer are switched off before installing the hardware.

2.3 Installing Outlet valve and Fraction collector (optional)

General

The Outlet valve PV-908 and Fraction collector Frac-901 are optional and not mounted at the factory. The installation of these components is desc ribed in the ÄKTAbasic 10 User Manual, Section A.5.

2.4 Installing Autosampler A-900 and the XT components

General

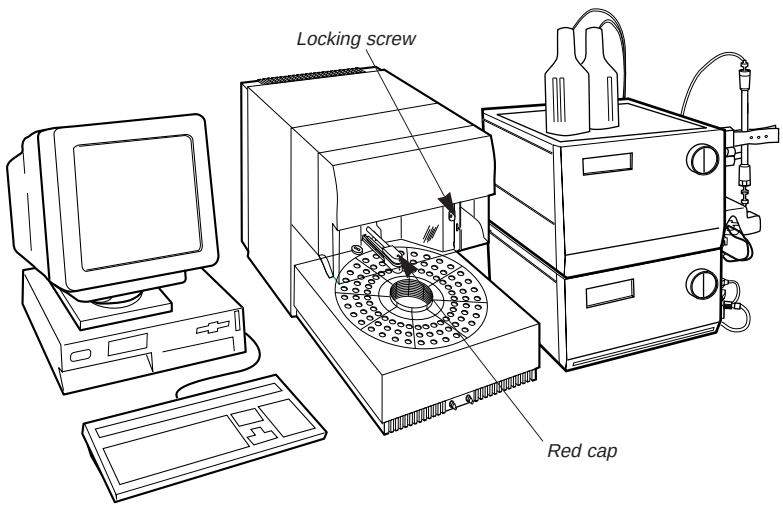
The A–900 needs approximately 28 cm of bench space and a mains connection of 220-240 V or 100-120 V, 50/60 Hz, preferably taken from the ÄKTAbasic 10 base platform.

The recommended position is to the left of the separation equipment of ÄKTAbasic 10, with approximately 3 cm spacing.

The A-900 has two transportation safety devices which must be removed:

- Remove the locking screw holding the front cover. It is located at the right side of the front cover. Save the locking screw.
- A red plastic cap is attached over the needle washing mechanism below the front cover. Remove the red plastic cap and save it.

See also the installation instructions given in the Autosampler A-900 User Manual, Chapter 2.

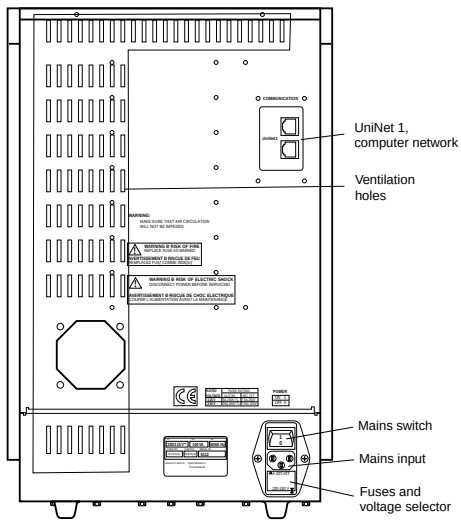


Connecting to the UniNet-1 communication network

CAUTION! The mains power to ÄKTAbasic 10 and the computer must be switched OFF before disconnecting or connecting the *UniNet-1* cables.

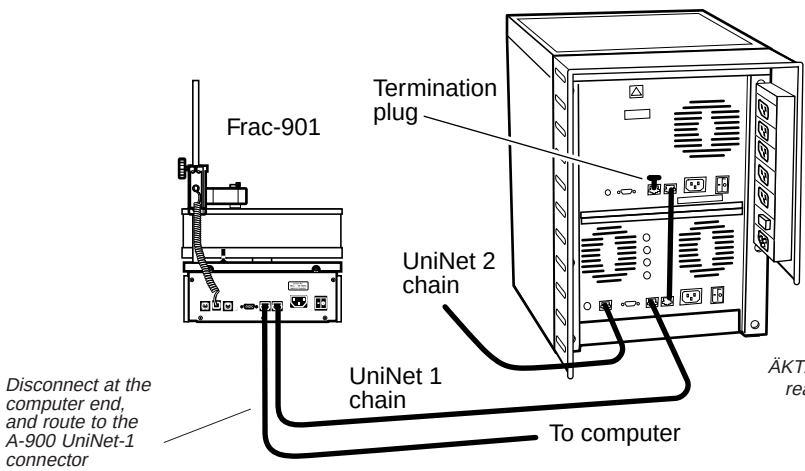
A-900 is controlled from a PC running UNICORN version 3.0 or higher using *UniNet-1* cables.

Autosampler A-900 rear panel

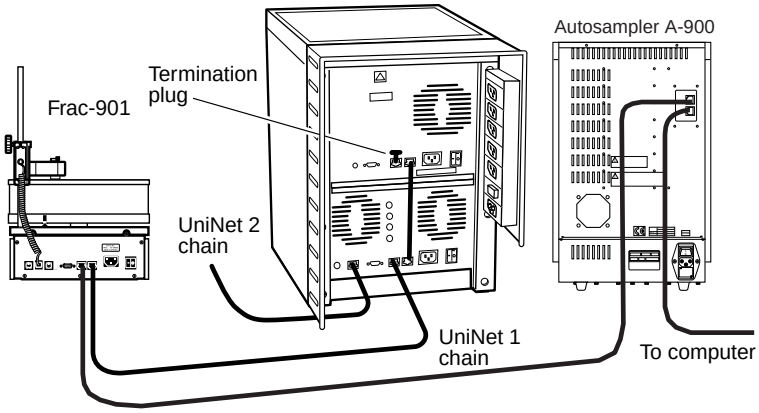


1 Disconnect the UniNet-1 cable running between Frac-900 and the computer, in the computer end.

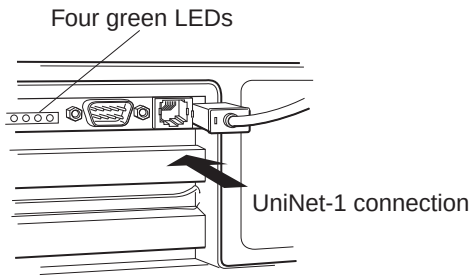
Note: *If no fraction collector Frac-900 is used, disconnect the UniNet-1 cable running between the Pump P-900 and the computer.*



2 Connect this UniNet-1 cable to any of the two UniNet-1 connectors in A-900.



3 Connect a new UniNet-1 cable (supplied with the A-900) from the other UniNet-1 connector on the A-900 to the computer. The UniNet-1 connection to the computer must be made to the board with four green LEDs.



Connecting A-900 to mains voltage

WARNING! A-900 must be connected to a grounded mains socket.

WARNING! For continued protection against risk of fire, replace only with fuses of specified type and rating. See Technical specifications in the A-900 User Manual for fuse data.

Before connecting the mains cord to the ÄKTAbasic 10 base platform, check that the voltage setting of the A-900 matches the local mains supply voltage and main fuses. Use the supplied appliance with protective grounding.

If the indicated voltage is not correct, select the proper voltage by removing, inverting, and then re-inserting the voltage selector cartridge. Check that the corect fuses are installed. If not, replace them with fuses as stated below:

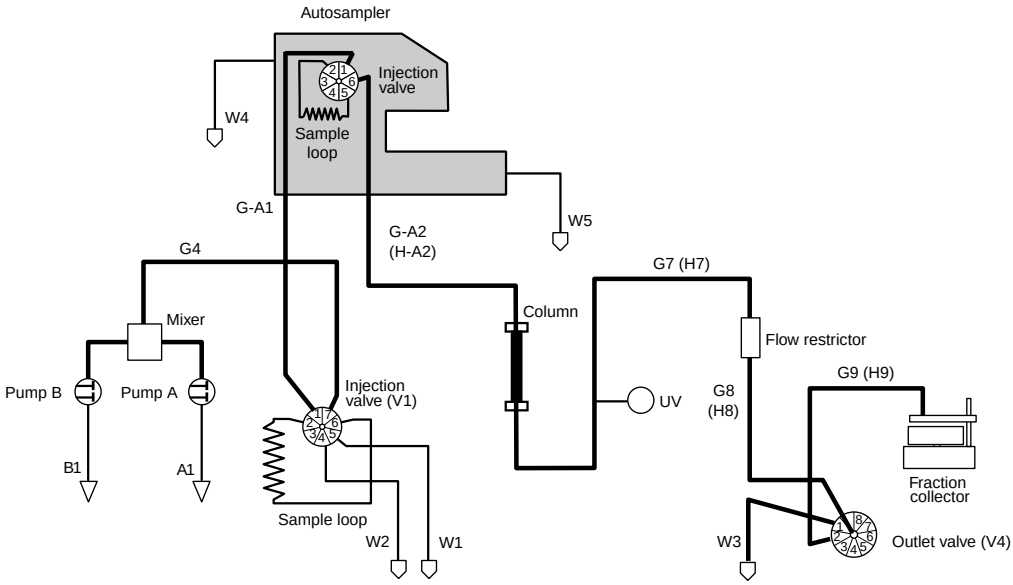
- For 110-120 VAC, use two 5 AT-fuses (slow).
- For 220-240 VAC, use two 2.5 AT-fuses (slow).

When the voltage selection and fuses are correct for the local mains power source, plug in the power cord in a free outlet in the base platform of the ÄKTAbasic 10 separation unit.

CAUTION! DO NOT switch on any of the equipment yet!

Connecting capillaries between A-900 and ÄKTAbasic 10

The tubings to be connected between A-900 and the ÄKTAbasic 10 separation unit are included in ÄKTAdesign 10XT Tubing kit 18-1122-65. The flow diagram below shows the location of A-900 in the ÄKTAbasic 10 flow path.



1 Remove the capillary connecting the Injection valve with the Column top inlet.

2 Select the capillaries matching the tubing kit to be used in ÄKTAbasic 10 after the column. Use the capillaries marked G-A1 and G-A2 if you intend to use the 0.25 tubing kit, and the capillaries marked G-A1 and H-A2 if you intend to use the 0.50 tubing kit.
See ÄKTAbasic 10 User Manual, Section 3.3 for general recommendations on selecting column and tubing kit.

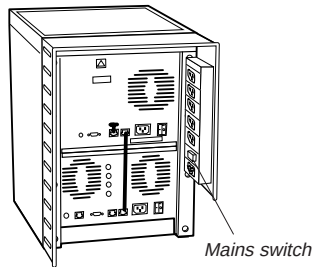
3 Both capillaries are routed in the space between the UV monitor UV-900 and the pH/Conductivity monitor pH/C-900.

4 Connect the capillary marked G-A1 (l=750 mm) from the tubing kit between the ÄKTAbasic 10 Injection valve port 1 and the A-900 Injection valve port 1 (marked “pump”).

5 Connect the capillary marked G-A2/H-A2 (l=800 mm) from the tubing kit between the A-900 Injection valve port 6 (marked “column”), and the UV flow cell inlet.
Note: *This connection is only temporary. When the installation test is successfully performed, G-A2 (H-A2) is connected to the top inlet of the column, and the capillary connecting the column outlet and the UV flow cell inlet is refitted .*

6 Check additional internal and waste tubing connections on A-900 according to the A-900 User Manual instructions.

Preparing for the installation test

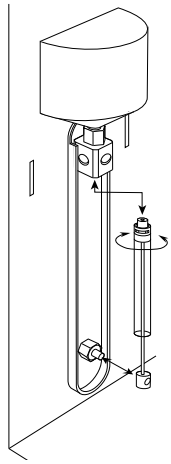


1 Switch on A-900 at the rear panel. Then switch on the ÄKTAbasic 10 chromatography system with the mains switch located at the rear.

2 Switch on the computer and the printer.

3 Perform a login procedure (see Chapter 2 in "Making your first runs"), press the **Control System** button, and select **System:Settings:Special**.

4 Check that the default sample loop volume (100 µl), syringe volume (250 µl) and needle height (2 mm) are selected.



5 Manually, run the **SyringeEnd** instruction under **System Control:Manual: Flowpath:AutosamplerControl**

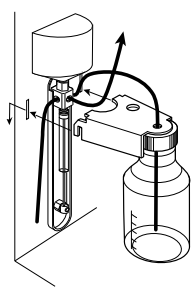
6 Remove the syringe from the syringe valve. Make sure that the Luer connector in the valve remains in place.

7 Fill the syringe with with 20% isopropanol in water from a vessel.

Note: *Methanol can be used instead of isopropanol.*

8 Replace the filled syringe. Fully tighten the syringe so that a gap between the plunger tip and the syringe end is obtained after having executed the **SyringeHome** instruction under **System Control:Manual: Flowpath:AutosamplerControl**.

Note: *If the syringe holder is not fully tightened, i.e. no gap is obtained, the plunger tip moves forcefully into the syringe end which can damage the syringe.*



9 Fill the wash solvent bottle with 20% isopropanol in water. If the sample contains large amounts of salt, or if the sample is very sensitive to isopropanol, use water as wash solvent instead (degas and change the water every day!).

10 Screw the bottle into the wash solvent holder and place the holder on the A-900.

11 Put the wash solvent tubing in the wash solvent bottle.

12 Fill the tubing using the **SyringeEnd** and **SyringeHome** instructions.

SyringeEnd aspirates a syringe volume of wash solvent from the wash solvent bottle and fills the wash solvent tubing.

SyringeHome dispenses the syringe contents to the syringe waste.

Repeat this action a number of times until the wash solvent tubing and the syringe are completely filled.

13 After the wash solvent tubing and the syringe are filled, use the **InitialWash** instruction to perform a standard wash. All tubing connected to the syringe valve will be filled and flushed with wash solvent.

Purging the syringe in A-900

1 Manually, run the **SyringeEnd** and **SyringeHome** instructions alternately.

2 Repeat until no more air is left in the syringe (more than 5 repeats can be necessary).

3 Finish by performing the **InitialWash** instruction.

Checking the needle height in A-900

1 Remove the tray segment next to (on the left side) the one directly below the needle arm.

2 Put a vial with a flat bottom, filled with e.g. water, in the tray segment directly below the needle arm. The bottom of the vial must be clearly visible.

3 Carry out a manual injection by issuing the **System Control:Manual:Flowpath:InjectionFlushed** instruction.

4 Check visually that the needle stops approximately 2 mm above the vial bottom.

5 If not, go to **System:Settings:Special** to adjust the needle height. The default setting is 2 mm.

Note: *Check the needle height with 2 mm as default setting whenever the needle is replaced. The 2 mm setting is only a relative measure to ensure that the needle stops above the vial bottom regardless of which type of vial used. Adjust the final setting in small steps until a needle height suitable for the actual use is obtained (type of vial, sample volume available, etc.).*

2.5 Installation test

General

The installation test performs a total check of the liquid processing parts of the ÄKTAbasic 10 separation unit. This is to establish that the integration of the additional parts in ÄKTAbasic 10 XT upgrade kit has not disturbed the total system function.

Preparation

1 Verify that the Pump P-900 is primed and purged according to the instructions in the ÄKTAbasic 10 User Maual.

2 Prepare a flask containing 500 ml of 0.4% acetone in water solution.

3 Fill two new standard vials with this solution and seal them properly with the correct type of cap or septum.

4 Put one vial in position 1, and the other vial in position 2 in a standard tray.

5 Immerse the inlet tubing B1 into the rest of the 0.4% acetone solution.

6 Immerse the inlet tubing A1 into a flask containing distilled water.

7 Click on the **Main menu** button in the Task bar.

Main menu button

8 Select **File:Printer setup...** from the menu bar. Select the appropriate printer from the list and click on **Job properties....** Under **Page Orientation**, select **Landscape**, then click on **Save**. Click on **OK** to acknowledge the printer chosen.

9 Click on the **Instant Run** button .

10 Select the method **Insttest** in the templates list and make sure that **Any** is selected for both **Technique:** and **Column:**.

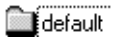
11 Click on **Run**.

12 When the **Method** window is shown, click on **Next**, and then **Next** again.

13 Click on **Start** to start the Installation test. The run time is approximately 30 minutes.

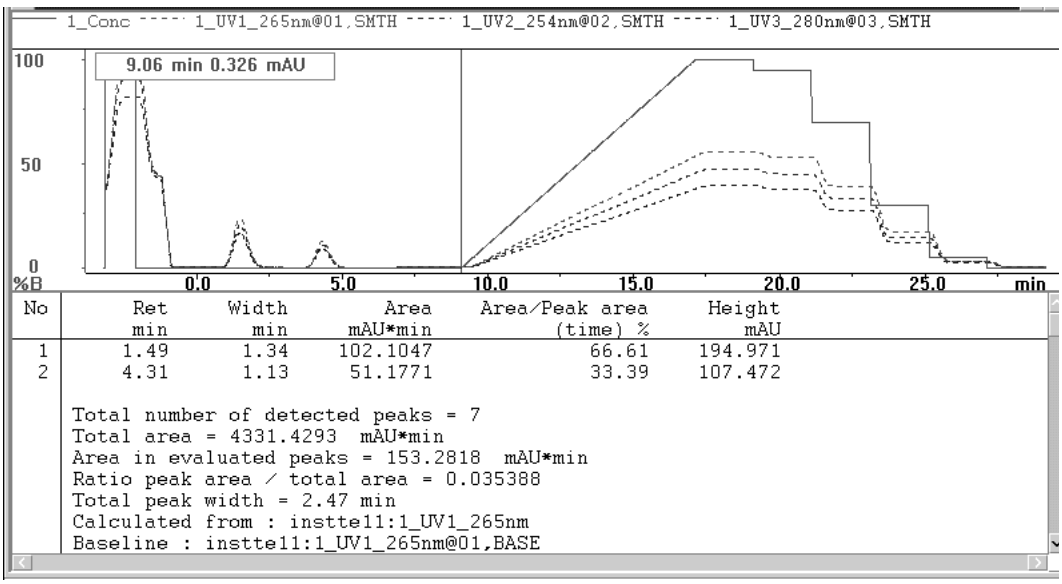
Evaluating the installation test result

1 Click on the **Main menu** button in the Task bar.

2 Double-click on 

in the results panel and then double-click on the **Instte01** icon to open the result file.

3 Maximise the chromatogram window by clicking on in the upper right corner.



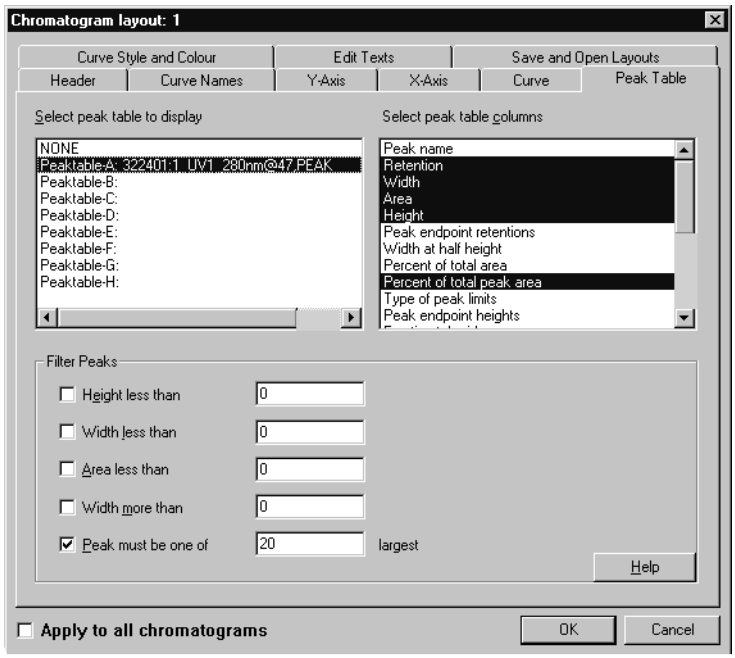
No	Ret min	Width min	Area mAU*min	Area/Peak area (time) %	Height mAU
1	1.49	1.34	102.1047	66.61	194.971
2	4.31	1.13	51.1771	33.39	107.472

Total number of detected peaks = 7
Total area = 4331.4293 mAU*min
Area in evaluated peaks = 153.2818 mAU*min
Ratio peak area / total area = 0.035388
Total peak width = 2.47 min
Calculated from : instte11:1_UV1_265nm
Baseline : instte11:1_UV1_265nm@01,BASE

4 Click in the chromatogram window with the right mouse button, and select **Properties.** De-select the first ten curves and then check that the following curves are displayed:

11 **instte01:1_UV1_265nm@01,SMTH**
13 **instte01:1_UV2_254nm@02,SMTH**
14 **instte01:1_UV3_280nm@03,SMTH**

5 Click on the **Peak Table** tag. Check that **Peak Table A** is selected and select "Percent of total peak area" in the **Peak Table Column**. Press **OK**.



6 Double-click on xy in the upper left corner of the chromatogram window and read the absorbance (in mAU) for the steps corresponding to **instte01:1_UV1_265nm@01,SMTH** by dragging the vertical bar to the constant section of each plateau.

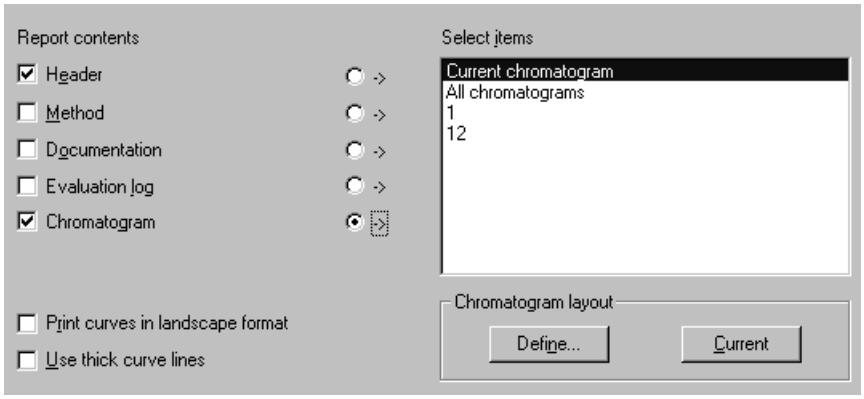
Enter the absorbance values (in mAU) in column 2 in the **Step response table** of the Test record, leaving out the decimals.

7 Read the absorbance for the plateaus corresponding to 0% and 100% B for the curves:

11 instte01:1_UV1_265nm@01,SMTH
13 instte01:1_UV2_254nm@02,SMTH
14 instte01:1_UV3_280nm@03,SMTH
and enter the values in column 2 in the UV **response table** of the Test record.

Change the curve to be measured by clicking on xy . The colour of the value shown corresponds to the colour of the curve selected.

8 Select **File:Report...** and select the boxes **Header** and **Chromatogram**. Under **Report contents**, click on the radio button to the right of the **Chromatogram** selection.



9 Under **Select items**, highlight **Current chromatograms**.

10 Click on **Define...** and select only the following curves to be included in the report:

06 instte01:1_Conc.
11 instte01:1_UV1_265nm@01,SMTH
13 instte01:1_UV2_254nm@02,SMTH
14 instte01:1_UV3_280nm@03,SMTH
Then click on **OK**.

11 Click on **Print** to obtain a printed report of the results.

Evaluating the gradient

1 Place a ruler along the gradient part of curve **instte01:1_UV1_265nm@01,SMTH** in the printed report.

2 The curve should be linear between 10% B and 90% B and void of discontinuities.

3 Fill in the Test record.

Evaluating the step response

1 Calculate the relative adsorption plateau heights for curve **instte01:1_UV1_265nm@01,SMTH** in the following way:

Subtract the base line value (0% B) from each of the values in column 2 in the Step response table of the Test record. Enter the results in column 3.

Divide each value in column 3 by the base line corrected value corresponding to 100% B. Multiply by 100 and enter the results in column 4.

2 The values of column 4 should all fall within the intervals given in column 5.

Evaluating the UV response

1 Calculate the UV response ratios in the following way:

Subtract the base line values (0% B) corresponding to each UV curve from the values in column 2 of the UV response table of the Test record. Enter the results in column 3.

Calculate the absorbance ratios 265 nm/254 nm and 265 nm/280 nm using the values from column 3 and enter the results in column 4.

2 The ratios obtained should all fall within the intervals given in column 5.

Evaluating the Autosampler peaks

1 In the printed report, read the Area and Area/Peak area % values for peaks No. 1 and 2.

2 Fill in the Test record.

3 Check that the values fall within the given intervals.

The installation procedure is completed when all results fall inside the limits specified in the Test record.

Note: *Remember to connect the G-A2 (H-A2) capillary to the top of the column, and the capillary from the column outlet to the UV flow cell inlet.*

Correcting faulty evaluation results

Faulty Gradient

- The gradient is linear but the interval is to small. The mixer chamber is too large, or the mixer is faulty.
- Disturbances. These may arise from air in the pump. Purge again and repeat the test run. If persistent, faulty check valves or bad piston sealings in the pump may be the cause. Refer to the Pump P-900 User Manual.

Faulty step response

- All values are faulty. This may arise from air in the pump or a faulty pump. Purge again and repeat the test run. If persistent, a faulty pump may be the cause. Refer to the Pump P-900 User Manual.
- 5% and 95% faulty. This may be due to bad sealings in the pump (5% faulty=pump module B, 95% faulty=pump module A). Refer to the Pump P-900 User Manual.

Faulty Autosampler peak values

- If values are faulty, make sure that the A-900 is properly purged and that all tubing fittings are properly tightened.

2.6 Test record

Gradient test result

Gradient linear from ..... % B to ..... % B. (10 – 90 %)

Step response test result

Step response table:

1 Programmed Conc. %B	2 Value read (mAU)	3 Base line corrected value	4 Normalised value	5 Allowed interval
100				
95				94 - 96
70				69 - 71
30				29 - 31
5				4 - 6
0				

UV response test result

UV response table:

1 Wavelength (nm)	2 Value read		3 Base line corrected value	4 Absorbance ratio	5 Allowed interval
	100% B	0% B			
254					
265 /254					1.14 - 1.24
265					
265 /280					1.32 - 1.42
280					

Autosampler peak test result

Autosampler peak table:

1 Peak no.	2 Area (mAU x min)	3 Area/Peak area (%)	4 Allowed interval (%)
1			66.1 - 67.2
2			32.8 - 33.9

3 Operation

3.1 General

A-900 is controlled from a PC running UNICORN version 3.0 or higher. It cannot be used as a stand-alone module. A-900 can be controlled using the B10X** strategy, either automatically from a template method, or manually via commands.

The A-900 can be used for automatic sample injection from a few microlitres to one millilitre of sample solution. To achieve optimal performance, selection of sample loop volume, syringe size and speed, plus the washing method between injections must be considered.

The sample injection techniques, which can be programmed in the method editor and then utilised in automatic runs, are as follows:

- **InjectionFlushed**: The sample loop is completely (quantitatively) filled with sample resulting in extremely good reproducibility.
- **InjectionPartial**: The sample loop is partially filled with sample giving low sample loss and allowing programmable injection volumes.
- **InjectionPickup**: After aspiration from the vial, the sample volume is transported into the loop with transport liquid (mobile phase) from another vial resulting in virtually no sample loss.

These sample injection techniques can also be ordered manually from **System Control:Manual:Flowpath**

Manual functions used for setting up and maintaining the A-900 are found in:
System Control:Manual:Flowpath:AutosamplerControl.

The available commands are as follows:

- **InitialWash**
- **RotateTray**
- **SyringeHome/End**
- **NeedleHome/Front**

Parameters for setting the syringe speed is found under
System:Settings:Special:AutosamplerParameters

All of these functions are described in the following sections. A table showing all the hardware related parameters for A-900 is given at the end of chapter 3.

3.2 InjectionFlushed

The **InjectionFlushed** instruction selects the flushed loop injection mode for A-900.

The syringe dispenser aspirates a flush volume (see also section 3.10) of sample into the sample line. The A-900 injection valve is turned to LOAD position, and the sample loop is filled with 1.5 - 3 times the sample loop volume (depending on the loop volume). The injection volume equals the loop volume. When the sample loop size is changed, the loop volume setting must be changed in **System:Settings** (100 µl is the default loop volume).

After each injection, the needle is rinsed:

- If **WashExt=NO** is selected in the method, the needle is only washed internally.
- If **WashExt=YES** is selected in the method, the needle is also washed on the outside.

In both cases, the buffer tubing is also washed.

An air segment (**Air segment=Air**) is recommended to reduce the amount of the flush volume. The air segment is not injected.

More details of flushed loop injection are found in the Autosampler A-900 User Manual, Reference information A.

3.3 InjectionPartial

The **InjectionPartial** instruction selects the partial loopfill injection mode for A-900.

The syringe dispenser aspirates a flush volume (see also section 3.10) of sample into the sample line. The A-900 injection valve is turned to LOAD position, and the sample loop is filled by transporting the selected injection volume into the sample loop, which is partially filled with sample.

After each injection, the needle is rinsed:

- If **WashExt=NO** is selected in the method, the needle is only washed internally.
- If **WashExt=YES** is selected in the method, the needle is also washed on the outside.

In both cases, the buffer tubing is also washed.

An air segment (**Air segment=Air**) is recommended to reduce the amount of the flush volume. The air segment is not injected.

More details of partial loopfill are found in the Autosampler A-900 User Manual, Reference information A.

3.4 InjectionPick-up

The **InjectionPickup** instruction selects the µl pick-up injection mode for A-900.

The syringe dispenser aspirates transport liquid from the transport vial into the sample line. The A-900 injection valve is turned to LOAD position. The needle moves from the transport vial to a sample vial and aspirates the selected injection volume. The needle moves back to the transport vial and the sample is quantitatively transported into the sample loop with transport liquid.

After each injection, the needle is rinsed:

- If **WashExt=NO** is selected in the method, the needle is only washed internally.
- If **WashExt=YES** is selected in the method, the needle is also washed on the outside.

In both cases, the buffering tubing is also washed.

An air segment is not recommended for this injection mode (**Air segment=NoAir**) because the air segment at the front of the sample plug is injected.

More details of µl pick-up are found in the Autosampler A-900 User Manual, Reference information A.

3.5 InitialWash

The **InitialWash** instruction performs a complete wash of the needle and the syringe.

The syringe is first washed using one syringe volume.

Then the needle is moved to its home position and washed internally.

Finally, the needle moves to its front position and washed both internally and externally.

3.6 RotateTray

The **RotateTray** instruction is used to rotate the tray, e.g. when new vials are loaded into the tray segments.

3.7 SyringeHome/End

The **SyringeHome/End** instructions are used when the syringe is replaced. They are also used for purging the syringe, i.e. to remove air.

SyringeHome empties the syringe contents to the syringe waste container.

SyringeEnd aspirates wash solvent into the syringe from the wash bottle.

Note: *When the performed action is completed, make sure the syringe is run to the Home position before leaving the manual function.*

3.8 NeedleHome/Front

The **NeedleHome/Front** instructions are used when the sample needle is replaced.

NeedleHome moves the needle to its normal position.

NeedleFront moves the needle closer to the front of A-900.

3.9 AutosamplerSyringeSpeed

The **AutosamplerSyringeSpeed** parameters are used to manually preset the speed of the syringe. The syringe speed needs adjusting when changing syringe size, using highly viscous samples, or using volatile eluents.

Three different speed settings are available:

- Low.
- Normal (default setting).
- High.

The actual speed is dependent on the speed setting and a scaling factor, and on the syringe volume. The scaling factor range of 0.1 - 1.0 is used to reduce the basic speed setting. Speed setting=low and scaling factor=0.2 means that low speed is reduced to 1/5 of original low speed.

Syringe volume	*Viscosity*	*Speed setting*	*Scaling factor*
250 µl	Normal	Normal	1.0
250 µl	High (5 – 10 cP)	Low	0.2
500 µl	Normal	Low	1.0
1000 µl	Normal	Low	1.0

Note: *If the sample is viscous, the flush volume can be enlarged and the syringe speed reduced to enhance performance.*

3.10 Hardware related parameters

All of the following parameters are found under:
System:Settings:Special:AutosamplerParameters

Parameter	*Value*	*Comment*
LoopVol	10-1000 µl (100 µl is default)	Sample loop volume
SyringeVol	100, 250, 500 or 1000 µl (250 µl is default)	Syringe volume
FlushVol	30-100 µl (40 µl is default)	Flush volume. The amount of sample, or transport liquid, taken from a vial before the loop is filled with sample.
WashVol	300-9999 µl (300 µl is default)	Wash volume. Sets the volume for the external wash (WashExt) programmable in the different injection modes.
TrayType	StandardVial_A MicroVial_D	A=Standard vial O.D. 12 mm. D=Micro vial O.D. 7 mm
HeadPress	No, Yes	Head-space pressure. Applied via the outer air needle. Assures that no air or vapour bubbles are formed during sample aspiration.
NeedleHgt	0-40 mm (2 mm is default)	Needle height. A relative measure used to adjust the space between the needle and the bottom of the vial used.
AutosamplerSyringeSpeed	Low Normal High (Normal is default)	Syringe speed setting.
Scaling	0.1-1.0 (1.0 is default)	Syringe speed scaling factor.

4 Maintenance

The ÄKTAbasic 10 XT upgrade kit items require no periodic maintenance.

Autosampler A-900 specific maintenance requirements are described in the Autosampler A-900 User Manual, chapter 4.

4.1 Cleaning

A-900 should be kept clean and spilled liquid should be wiped off before it dries. The front cover should always be folded down when access to the internal parts is not necessary.

SHARP NEEDLE
AND MOVING PARTS
KEEP HANDS CLEAR

The module should be wiped regularly with a damp cloth. Allow to dry completely before use.

5 Trouble shooting

5.1 Faults and actions

If the suggested actions do not correct the fault, call Amersham Biosciences.

WARNING! Always disconnect the power supply before attempting to replace any item during maintenance.

WARNING! The modules must not be opened by the user. They contain high voltage circuits which can deliver a lethal electric shock.

CAUTION! Only spare parts approved or supplied by Amersham Biosciences may be used for maintaining and servicing the modules.

Fault	*Possible cause and Action*
Ghost peaks at high pressure	1 Bad capillary connections. Replace connectors if necessary.
Backpressure too high	1 Capillaries too narrow. Change to 0.50 tubing kit.
Sample needle bent, no injection performed	1 Wrong type of septa, deformed septa or needle height set too low. Replace needle and re-adjust needle height if necessary.
Needle destroyed in injection using micro vial segments	1 Segments badly oriented or septa bent down. Replace needle. Check segments and replace septa.
Air bubbles	1 Wrong needle height. Adjust the needle height. 2 No wash solution or not properly purged syringe. Check the wash solvent bottle and/or purge the syringe. 3 Syringe speed too high. Lower the syringe speed.
Bad reproducibility	1 Injection valve switched dry. Replace rotor seal. 2 Syringe valve coupling leaking. Check and tighten coupling. Replace if necessary. 3 Syringe leaking problem. Replace syringe. 4 Peaks of different height. Connectors not tightened in the injection valve. Tighten connectors. 5 Air in the syringe. See “Air bubbles” above.

Fault	*Possible cause and Action*
Error message during initial wash	1 Syringe valve not switched properly. Check syringe installation. Refit if necessary.
Error message during other commands	1 Syringe in End position. Issue a **SyringeHome** command. 2 A-900 is not ready to receive a command. Wait a while and try again.

Reference information

A Description

A.1 UNICORN version 3.0

UNICORN is a control system for real-time control of chromatographic separation systems from a personal computer. UNICORN operates together with the ÄKTAbasic 10 chromatography system. It runs under the operating system Windows™NT.

UNICORN version 3.0 software enables the use of UNICORN Analysis module software.

A.2 UNICORN Analysis module

UNICORN Analysis module software extends the UNICORN Evaluation module with functions for quantification and molecular size calculation. It allows the user to determine the absolute quantity or concentration of a sample component and determine its molecular size.

The Analysis module is accessed in the Evaluation window under two new menus, **Quantitate** and **Mol. size.** The Analysis module uses many of the facilities within the Evaluation module.

The **Quantitate** function uses peak data from standards to produce calibration curves which can then be used to evaluate the amount and concentration of components in a sample.

The **Mol. size** function uses a molecular size curve prepared from one or more standards to determine the molecular size of components in a sample.

A.3 Autosampler A-900

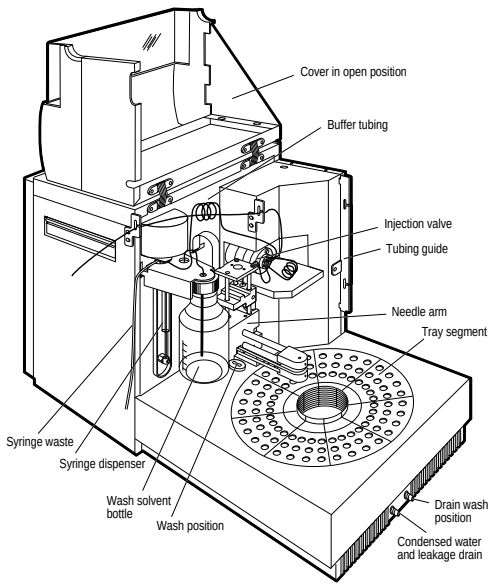
A sequence of valve switching and syringe dispenser controlled sample withdrawal enables the A-900 to inject sample volumes in a range from a few microliters to 1 ml in a simple and reproducible manner.

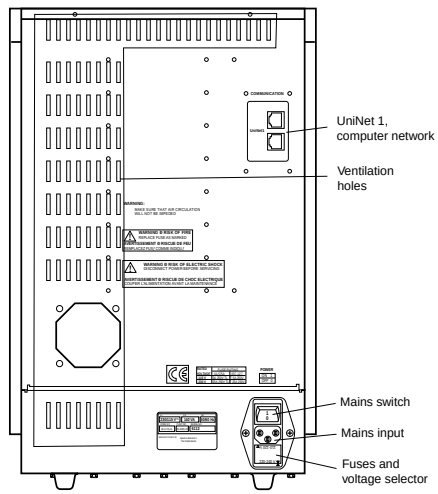
Three different injection methods can be selected:

- Flushed loop: The sample loop is completely (quantitatively) filled with sample resulting in extremely good reproducibility (better than 0.3%).
- Partial loopfill: The sample loop is partially filled with sample giving low sample loss and allowing programmable injection volumes.
- µl pick-up: After aspiration from the vial, the sample volume is transported into the loop with transport liquid (mobile phase) from another vial. This eliminates sample loss.

The A-900 uses a syringe to aspirate the sample from a vial into the sample loop. To prevent contamination of the syringe, the A-900 is equipped with a buffer tubing between the syringe and the injection valve.

An integrated washing mechanism removes the sample from the buffer tubing, and sample needle, and also rinses both components.





The A-900 contains no internal user replaceable items.

B Technical specifications

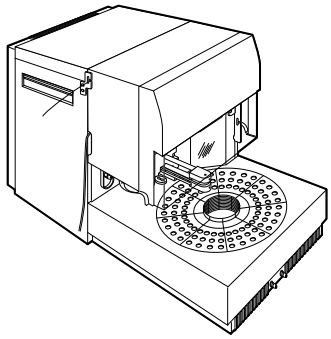
See Technical Specifications in the Autosampler A-900 User Manual and the ÄKTAbasic 10 User Manual.

C Accessories and spare parts

Item	Quantity per pack	Code no.
ÄKTAdesign 10 XT Tubing kit	1	18-1122-65
See also Accessories and spare parts in Autosampler A-900 User Manual		

November 1998

Short instructions



The following short instructions are intended as a guide to users who are fully familiar with the safety precautions and operating instructions described in this manual. The instructions assume that the equipment is installed according to the installation instructions.

A-900 is controlled from UNICORN. For method and manual control, use the instructions and parameters given in the following:

Method: **Method editor:Text instructions:Flowpath**
Manual: **System Control:Manual:Flowpath**

1 For Flushed loop injection, select the instruction **InjectionFlushed** and enter the sample vial number.

2 For Partial loopfill, select the instruction **InjectionPartial** and enter the sample vial number and injection volume.

3 For µl Pick-up, select the instruction **InjectionPickup** and enter the sample vial and transport liquid vial numbers and the injection volume.

Manual: **System Control:Manual:Flowpath:AutosamplerControl**

4 To wash the needle and syringe, use the instruction **InitialWash**.

5 To rotate the tray segments, use the instruction **RotateTray**.

6 To purge the syringe, use the instructions **SyringeHome** and **SyringeEnd** alternately to move the syringe plunger.

7 To change the needle, use the instructions **NeedleHome** and **NeedleFront** to move the needle.

Settings: **System:Settings:Special:AutosamplerParameters**

8 To set the relative needle height, use the **NeedleHgt** parameter.

9 To adjust the syringe speed, use the **AutosamplerSyringeSpeed** and **Scaling** parameters.

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